PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Roberto R. Panepucci et al.	Examiner: Michael Stahl
Serial No.:	10/781,339	Group Art Unit: 2874
Filed:	February 18, 2004	Docket: 1153.099US1
Title:	OPTICAL WAVEGUIDE DISPLACEMENT SENSOR	

DECLARATION UNDER 37 C.F.R. § 1.131

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This declaration is submitted under 37 C.F.R. §1.131 in response to rejection of U.S. Patent Application Serial Number 10/781,339 to establish invention of the subject matter of the rejected claims.

We, Roberto R. Panepucci, Bojan (Rob) Ilic, Michael Lipson and Vilson Rosa de Almeida, declare and say as follows:

1. We are the inventors of the subject matter in the above-identified U.S. Patent Application. We make this declaration to establish conception and/or reduction to practice of the invention claimed in the above-identified application in the United States at a date prior to July 23, 2003.

2. The USPTO Office Action mailed November 23, 2005, uses U.S. Patent Application Publication No. US 2005/0018946, filed July 23, 2003, issued to Tran et al. and assigned to Lucent Technologies Inc., to reject claims 1, 2, and 5-30 pending in the above-identified patent application.

3. The invention claimed in the above-identified patent application was conceived in the United States prior to July 2003. A copy of a proposal sent to both NIH in February 2003 and to NSF in March 2003 is attached as Exhibit A.

4. Fabrication results following submission of the above proposals and prior to July 23, 2003 illustrating the formation of a suitable gap in a waveguide for forming a cantilever which could be released via a simple etch are provided in Exhibit B.

5. The inventors listed in U.S. Patent Application Publication No. US 2005/0018946 had access to the lab where the fabrication results occurred.

6. Following processing of the proposals for funding and obtaining fabrication results, an invention disclosure was completed and submitted, and we diligently assisted our patent counsel to prepare and file U.S. Pat. Application 10/781,339. The invention disclosure is attached as Exhibit C with the actual dates redacted. The application was filed less than 4 months from receipt of the disclosure by patent counsel.

7. We declare that all statements made herein of my own individual knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 11/09/06 By _____
Roberto R. Panepucci

Date _____ By _____
Bojan (Rob) Ilic

Date _____ By _____
Michael Lipson

Date _____ By _____
Vilson Rosa de Almeida

S/N 10/781,339

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Date Nov 13, 06 By _____
MICHAL ~~Michael~~ Lipson

Date _____ By _____
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Date _____ By _____
Roberto R. Panepucci

Date _____ By _____
Bojan (Rob) Ilic

Date _____ By _____
Michael Lipson

Date November 10, 2006 By _____
Vilson Rosa de Almeida

Sensors: Arrayed Optical Detection of Nanoscale Biomass

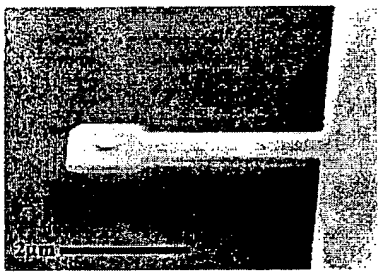
Summary:

The ability to detect small amounts of materials, including pathogenic bacteria and biomolecules integral to cell responses, is important for life science research, drug discovery, medical diagnostics and for homeland security. Most current detection technologies are either, bulky, expensive or slow. We propose a novel device/instrument for ultra-sensitive detection of molecular amounts of material with applications in pathogen and biochemical detection, medicine, drug discovery, and nanotechnology. The proposed technology allows the detection of an array of different targets in an intrinsically networked structure through fiber-optically linked structures. The technology lends itself to high throughput at low cost through use of disposable devices.

The device consists of a small all-silicon chip incorporating nano-mechanical cantilever resonators with novel silicon based passive integrated optics for motion detection. The principle of operation is based on highly-sensitive, immunospecific attachment of pathogens to silicon nanofabricated structures. In addition to detecting its presence, the mass of the pathogen is measured. Our preliminary results show that the cantilevers are able to detect mass in the attograms to picograms range. The additional information of the mass allows one to screen for false positives, greatly increasing the efficiency of the method.

The IC compatible fabrication technology allows the cost to be truly minimized for the disposable chip. Emerging from this method is the possibility of high resolution measurements of the dry mass of a great variety of pathogens, from large unicellular organisms to viruses and large macromolecules.

This novel Si-based nanophotonic motion detection scheme allows arrays of such devices to form a complete silicon platform for reduced cost, increased sensitivity and enormous system level integration revolutionizing functionality, cost and portability.



Nano-mechanical cantilever for mass detection (Cornell University)

1. Background

The current instrumentation for single molecule detection is largely based upon direct optical measurements which provide a limited ability to interrogate an unknown molecule. Spectroscopic techniques such as ultraviolet, IR and fluorescence all operate based upon the intrinsic spectral properties of the molecule alone or in combination with a label. The ability of these techniques to be informative is limited and this is especially true of molecules with a limited unique optical spectra and where there are a number of molecules in the milieu with similar spectral signatures (*i.e.* IR). In contrast, the cantilever based system will provide a second dimension in the analytical resolution of a molecule, the accurate measurement of its weight. While the test systems proposed here, bacteriophage are relatively large they will demonstrate the utility of the technology and suggest the likelihood of success for even more sensitive measurements of the mass of single molecules or their complexes.

The cantilever technology will be more sensitive than mass spectrometry and provide mass information for noncovalently linked complexes. Therefore, this technology will allow mass measurements on single complexes that are brought together by noncovalent linkages including van der Waals, hydrophobic and electrostatic interactions. Transcriptional complex

formation such as those that involve the assembly of the heat shock complex could be monitored with these cantilever devices. Interactions between protein subunits of cell signaling complexes could also be examined by tethering one member of the complex to the cantilever and allowing the other putative components to assemble.

Other methods of detecting biomolecules, including those that might be a biowarfare agent when attached to an antibody, using integrated optics have been demonstrated recently. However, these depend on tight fabrication tolerances due to the efficient coupling and single wavelength operation of the detection device, normally ring or disk resonators[1]. Also, these techniques require expensive accessories as single wavelength lasers are required, tuned to the fabricated device. In contrast, our scheme allows the use of inexpensive broadband sources, since wavelengths are selected in the chip.

1.1. Photonics - Si as a photonic material

Light can be confined by total internal reflection inside a high index of refraction medium surrounded by lower index of refraction. When such total internal reflection is achieved those optical rays can propagate with very little attenuation over large distances. This is the fundamental principle behind the field of fiber optics and integrated optics on chip.

Standard integrated optics platforms for biosensors are typically based on glass waveguides made on silica on silicon, diffused silica substrates or polymer waveguides.[2] Recently it was recognized that single crystal silicon is a very good candidate for integrated optics due to it's high refractive index and high transparency in the infra-red.[3,4]

High dielectric contrast confinement (e.g., the Si/SiO2 system) shrinks the wavelength of the light to dimensions of λ/n, where n is the refractive index of the core material. Photons in the 1.2-1.7 μm wavelength region can propagate in single mode transmission within silicon waveguides of less than 0.5 μm in cross section dimension due to its high index of refraction, n(Si) = 3.5. The fact that this is the standard wavelength range for the telecommunication industry has the advantage that components are readily available and have lower price. Furthermore, extremely sharp curves and bends can be produced in this system, allowing an unprecedented level of integration. Silicon-on-insulator as a photonic medium has unique advantages. Silicon's process maturity resulting from decades of microelectronic improvements, high material quality at affordable cost, and thermal conductivity are superior to other alternatives such as polymers, glasses and other semiconductor compounds.

1.1.1. Silicon-Micro-Ring Couplers for Wavelength Separation

The ability to make arrays of devices as proposed here is enabled by wavelength separation at the chip level. Ring resonators have become attractive for use in photonics due to their simple design and wavelength selectivity as compared with arrayed waveguide gratings (AWGs). Micron sized rings have been recently demonstrated in silicon-on-insulator waveguides.[5] Due to its high refractive index, silicon micro-ring resonators can be made smaller than 5 um in diameter, ensuring that the free-spectral range (FSR) of the device is larger than 30 nm, the erbium amplifier bandwidth, a relevant factor in telecommunications. Figure 1 shows a ring resonator fabricated in our group at Cornell. The devices were patterned by electron-beam lithography and subsequently etched by ICP-RIE following the same process and simultaneously with the nano-taper coupler described later.

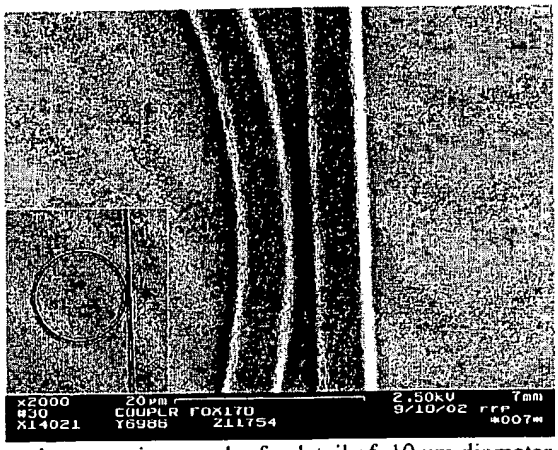
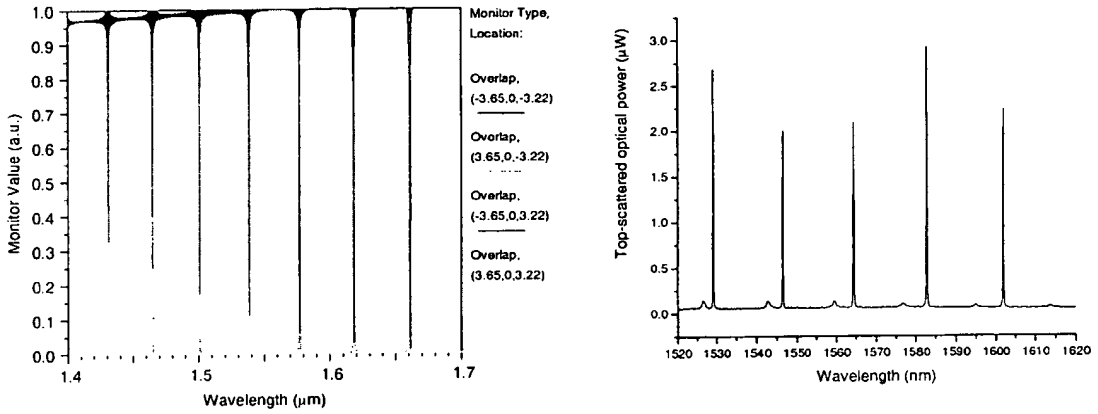


Figure 1 – Scanning electron micrograph of a detail of 10 um diameter ring coupled to the waveguide. Gap between waveguide and ring is approximately 150 nm. Inset shows the whole ring structure made out of single crystal silicon (scale bar applies to inset). Cornell University

Figure 2 (a) shows the simulated response of a single ring filter with a 5 um diameter silicon ring. Figure 2 (b) shows the measured spectra of the 10 μm ring-resonator seen in Fig.1. The spectra show the main TE polarized peaks and weaker TM polarized peaks.



(a) (b)

Figure 2 – (a) Simulated response from a single 5 um ring add-drop filter. (b) Measured response from the 10 um ring resonator. The scattered light intensity from the cavity was measured as a function of wavelength.

The FWHM of the peaks seen in the spectra are approximately 0.2 nm. This narrow response will enable the integration of a large number of devices (50-100) using single rings. With the use of pairs of coupled rings the free-spectral-range – the distance between the optical resonance peaks - can be increased by a factor of 2 or more, with a resulting increase in the number of addressable cantilever devices.

1.2. Micro-electro-mechanical system – MEMS

Micro-electro-mechanical systems (MEMS) are a generic denomination for miniaturized systems that use traditional tools from the microelectronics industry to create engineered structures with dimensions ranging from microns to millimeters. Perhaps one of the most

surprising tools that emerged from this technology is the atomic force microscope (AFM), which allows scientists now to detect single atoms on a crystal structure, or to investigate aspects of the physical structure of DNA and other large biological molecules. The principle of operation of the non-contact atomic force microscope is based on the variation of the attraction force with the distance to the surface. The measurable quantity detected in non-contact mode is the deflection or the change in frequency of oscillation of the AFM cantilever tip.

In recent years, micro and nanomechanical (MEMS and NEMS) oscillators[6-8] have attained a great deal of consideration since the practical significance of their exceptionally receptive properties open endless possibilities in the construction of a new class of resonant chemical[9,10] and biological[11] sensors. One of the simplest examples of mechanical oscillators is a cantilever of a rectangular shape, which can be easily simulated either analytically or numerically. Generally, biomolecular adsorption of target analytes to treated regions of a cantilever based sensors can alter mechanical stress within the cantilever and its total mass and thus influence the cantilever bending[12] and the cantilever natural frequency, respectively. Signal transduction is generally achieved by employing an optical deflection system[13] to measure the mechanical bending[12] or the frequency spectra resulting from additional loading by the adsorbed mass.[11]

We have successfully demonstrated a laboratory version of a resonance-frequency-based biological mass sensor, comprised of a low-stress silicon nitride (bulk micromachined) as well as stress free poly-silicon (surface micromachined) cantilever beams, and an optical deflection setup, for the detection of single *Escherichia coli* O157:H7 *(E. coli)*-cell-antibody binding events as well as selectively bound self assembled monolayers (SAMs). The shift in the resonance frequency of the fundamental mode of the cantilever beam was measured as a function of the additional cell loading and correlated to the mass of the specifically bound additional mass. The out of plane vibrational oscillations of the free end of the cantilever beam at and near the fundamental resonance frequency were detected using the both an optical deflection and interferometric systems. For the soft, bulk micromachined oscillators, the measured cantilever's vibrations was due solely to thermal mechanical noise in air (i.e., no drive signal was applied to the cantilever.)

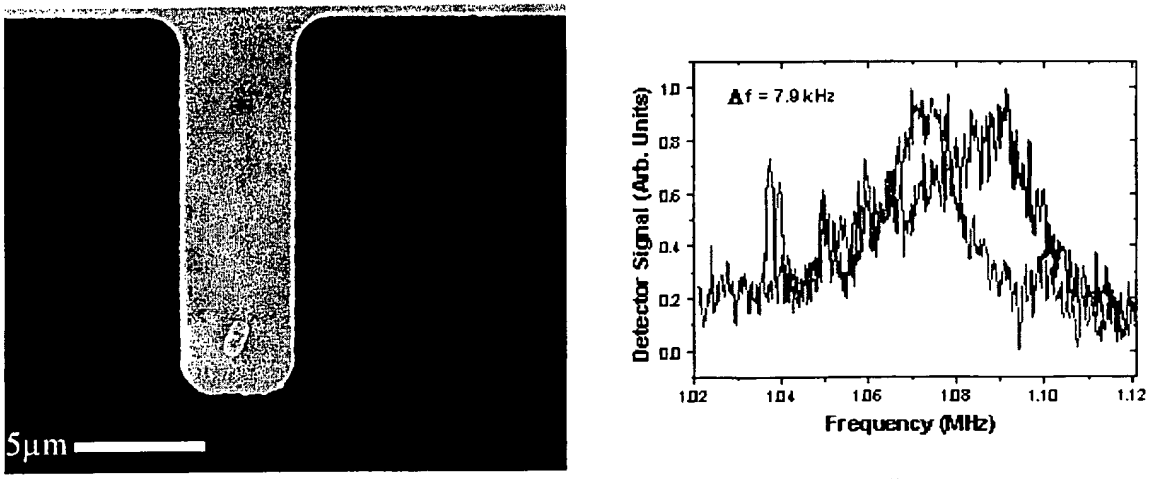
A single *E. coli* cell as well as single antibody monolayers were measured using the frequency shift approach (see Fig 3). The shift in the resonant frequency for a cantilever due to the additional mass loading is given by

$$\Delta f = 0.279\, m_{eff} \sqrt{\frac{E\, I}{l^3 m_o^3}} \qquad [1]$$

Here, I is the moment of inertia of the cantilever, E is the Young modulus of elasticity for the low stress silicon nitride (E_{measured} ~ 110GPa assuming a silicon nitride density of 3.4g/cm^3). $m_{eff} = m_{cell}\,(x / l,)$, where m_{eff} is the effective mass of a cell bound to the cantilever at a distance x from the fixed end (base) of the cantilever, m_{cell} is the mass of a cell, calculated from cell density and volume, estimated from AFM measurements, and m_o is the mass of the cantilever prior to cell attachment. (For a 15μm-long, 5μm-wide silicon nitride cantilever this formula suggests a mass sensitivity of 7.9 Hz/fg for mass added at the end of the cantilever, i.e., at $x = l$.)

A single *E. coli* cell[2] as well as single antibody monolayers were measured using the frequency shift approach (see Fig 3). The measured shift in the frequency was correlated to the mass of the cell (~665 fg) and is consistent with other reports and the above equation using an

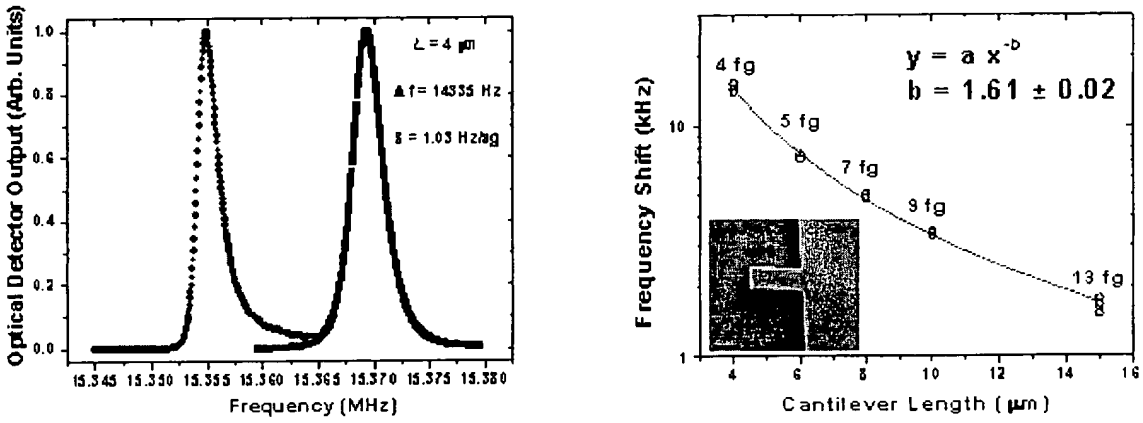
estimated volume of this cell. After fitting the data to a Lorenzian function, the observed shift in



(a) (b)

Figure 3 - (a) Scanning electron micrograph of an *E. coli* cell bound to immobilized antibody layer on a 5mm wide 15mm long cantilever. Respective cantilever vibrations before (black) and after (red) cell attachment with no external drive force. (Cornell University)

frequency was in good agreement with the expected shift from equation (1). Further verification using ANSYS, a finite element simulation module, showed a resonant frequency shift of approximately 10kHz. Our measurements demonstrate a correlation between a resonant frequency shift and single binding *E. coli* cell-antibody events. This technique is also useful for observing any substance able to be attached to the cantilever, making it a very sensitive balance.

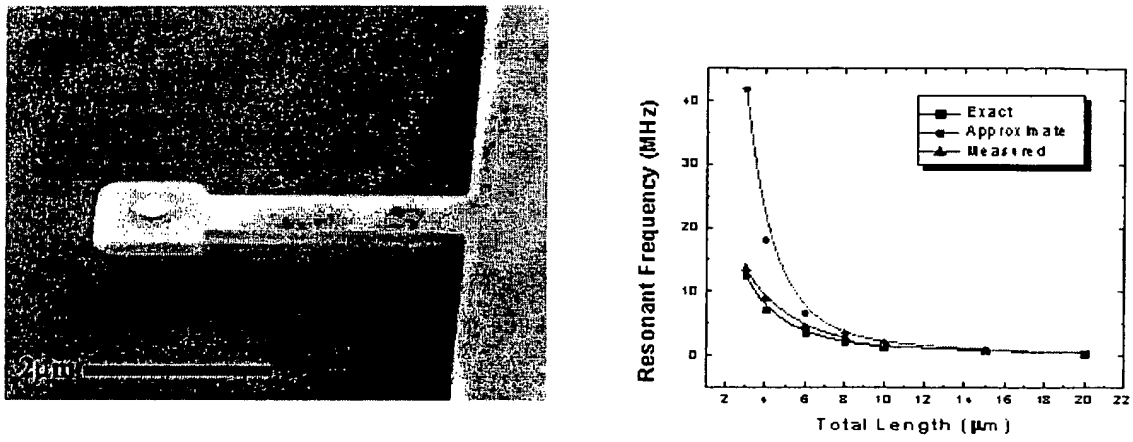


(a) (b)

Figure 4. Measured frequency spectra (•) before and (■) after the addition of the HMDS self assembled monolayer. (a) Frequency shift correspond to a mass of 4fg for the 4mm long cantilever.(b) Frequency shift for various cantilever dimensions. Polynomial fit indicates good agreement of the theoretically predicted frequency shift. (Cornell University)

To further extend the sensitivity of the oscillators, tailoring of device dimensions and vacuum encapsulation was employed. Figure 4 shows binding events due to the presence of Hexamethyldisilazane (HMDS) SAM. We have also detected the presence of

Aminopropyltriethoxysilane (APTS) and Octadecyltrichlorosilane (OTS) SAMs. From the resonant frequency shift, the mass of the adsorbed species is determined and compared to results obtained by other techniques.



(a) (b)

Figure 5. (a) Surface micromachined poly-silicon resonator with a gold anchor for selective binding of self assembled monolayers and bacteria. (b) Measured and calculated resonant characteristics for cantilevers of various dimensions. (Cornell University)

For the smallest device geometry (length = 4μm, width =1μm and thickness = 160nm), we observed a resonant frequency shift due to the presence of 4 femtograms of HMDS. We are currently investigating fabrication of cantilevers that allow selective binding at the free end (most sensitive area) of the oscillator (see Figure 5).

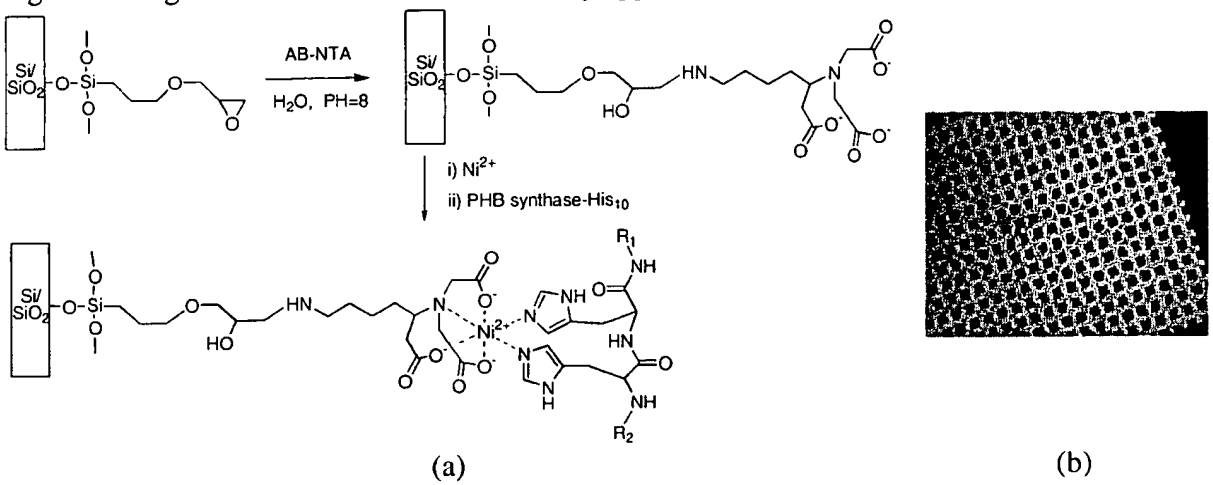
In all these examples, the detection is achieved through an externally collimated laser beam which is focused onto the apex of the cantilever and is reflected onto a split photodiode. The resonant peak of the AC signal extracted by a spectrum analyzer corresponds to the cantilever natural frequency. The measured out of plane resonant oscillations are either due to thermal and ambient vibrations or to various other external driving mechanisms.[14] The above described system has several drawbacks that are associated with a requirement of complex electronics to transduce the photodetector signal. Despite being hailed as a revolutionary detection technique, the external optical deflection has only achieved minor success in consumer sensing applications.

The ease of actuation and measurement of natural frequency of oscillators provides precisely the broad applications that modern sensors and actuators technology demands. The further infusion of such advanced systems into modern MEMS oscillators is necessary to overcome the limitations of the currently dominant measurement systems. The cause of this envisioned paradigm shift is the same as when tunneling currents, which monitored displacement and oscillations of AFM cantilevers, were replaced by the optical deflection technique. Our integrated optical detection method offers potentially significant performance and cost advantages over the conventional large-scale external optical deflection apparatus.

1.3. Surface Chemistries for Biosensing

Advances in the surface chemistries have promoted progress in a number of systems that integrate biological components with solid inorganic substrates. In addition to mixed self-assembled monolayer (mSAM) chemistries for the immobilization of proteins *via* primary amine

residues, nickel nitrilotriacetic acid (Ni-NTA) functionalized surfaces have been used to bind histidine (His)-tagged proteins. His-tags are fused proteins to facilitate purification from recombinant host systems. Direct attachment to silicon can be accomplished as suggested in Figure 6 using chemistries that we have already applied for enzyme immobilization.[15]



(a) (b)

Figure 6 – (a) Chemistries for enzyme immobilization in Si based substrates. (b) Gold covered silicon nitride treated with biotin terminated dendrimer chemistry. The surface was treated with streptavidin-ALEXA to reveal the uniform coverage of the pattern. (Cornell University)

In addition, a thiol-based chemistry can be employed to couple NTA to the surface of a thin layer of gold. This robust, selective and highly absorptive chemistry has also been demonstrated for binding kinetics of His-tag protein by SPR analysis.[16]

2. Technical Rational

2.1. System description

The proposed analytical chip is schematically depicted in Fig. 7. Light from and to the chip is carried to a measurement system through an optical fiber. The chip carrier contains a piezo-electric transducer to excite mechanical vibrations of the micro-mechanical cantilevers that perform the biosensing inside the chip.

The chip itself consists of a nano-taper input coupler leading to the main waveguide that distributes the optical signal. There are ring resonators coupled to the main waveguide that extract only a specific wavelength. Another waveguide extracts the optical signal from the ring resonator, and carries it to the cantilever waveguide which is suspended and free to oscillate. The cantilever waveguide has a sensitized region on its top part to which pathogens can bind. This extra mass at the tip of this cantilever causes a change of its natural oscillation frequency. In the preferred embodiment, a reflecting element is placed opposite to the waveguide. Alternative the signal can be collected by a receiving waveguide. This alternative approach will be considered later.

Operation of the sensor requires that initially the chip containing the cantilever, with a bio-sensitive site at its tip, be exposed to the medium containing the target. The target then binds to the cantilever and is permanently attached. The chip is then dried if a liquid medium is used, and measurements are taken.

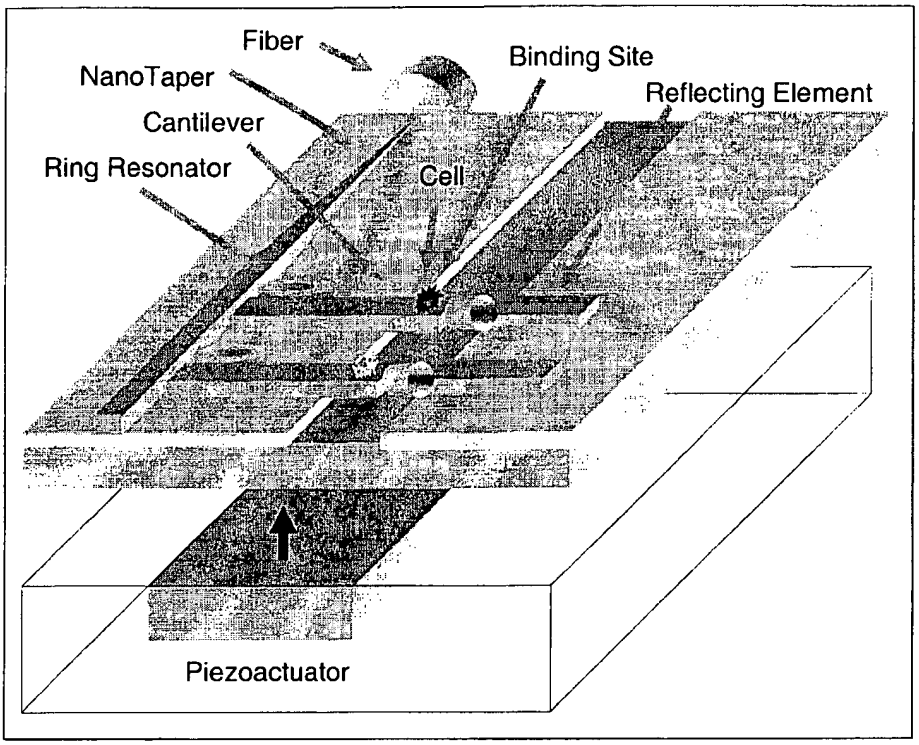


Figure 7 – Schematic diagram of chip with attached Fiber and Piezoactuator mount.

Optical power at different wavelengths is coupled from the fiber to the waveguides on chip. Light then propagates until it reaches the ring resonator tuned to its wavelength. Light is then transferred to the waveguide leading to the suspended section of the silicon waveguide that acts as a cantilever. At this point light exits the end of the cantilever and diffracts into free-space. A portion of this light is reflected back into the cantilever waveguide. The amount of light reflected back into the receiving waveguide depends strongly on the vertical deflection of the cantilever. The light coupled back into the waveguide has encoded in it, a modulation in intensity due to the mechanical oscillation of the cantilever. This light is coupled back through the ring resonator into the input waveguide, and exits at the edge of the chip through the nano-taper coupler into the fiber. The intensity of each optical wavelength is modulated by the oscillation of its respective cantilever. The optical signals reflected back into the fiber are carried by the fiber to a simple spectrometer that measures the oscillating signal of each cantilever. Because the ring resonator acts as a filter in and of itself, the input signal can be spectrally broad. Changes in temperature will not affect the device, as they do in other schemes that use rings and discs as active sensing area.

A piezoelectric actuator increases the intensity of the cantilever oscillation during successive frequency scans to optimize sensitivity and then the resonance frequency is determined with the mass being given by the shift in frequency. The change in mass due to the attachment of the target is determined by: $dm/m = -2\ df/f$. For measurements in vacuum this of the order of 10^3. This translates into a mass sensitivity range of attograms to picograms for the cantilevers we can manufacture.

2.2. Sub-System Requirements

The proposed system will require the development of a four major sub-systems. These are summarized in the list below. Each of the sub-systems requirements for the analytical chip is described in detail. Figure 8 shows a schematic of the analytical chip proposed.

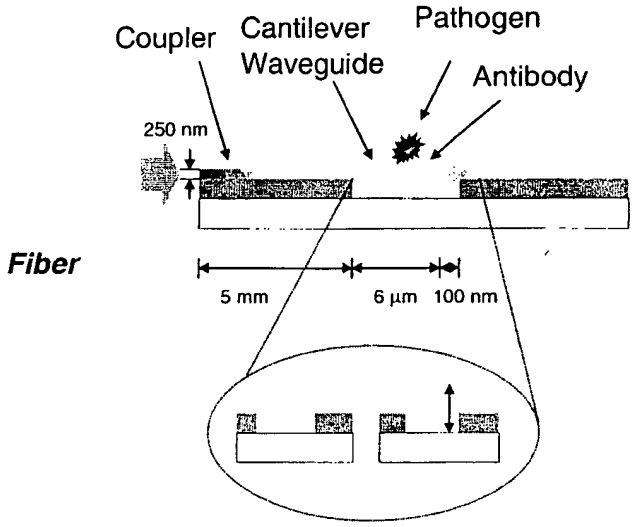


Figure 8 – Schematic of analytical chip with pathogen bound to antibody on top of cantilever waveguide. Also shown are the fiber source of light. The inset shows the cantilever in two states during oscillation.

Summary of key sub-systems:

- Fiber to waveguide coupling
- Silicon waveguide cantilever
- Ring Resonator for wavelength separation
- Bio sensitive site on cantilever

2.2.1. Fiber to waveguide couplers

Coupling to and from optical fibers to devices such as the analytical chip usually involves high losses due to mode-size and effective index mismatch. To date, most of the on-chip structures suggested to alleviate this coupling problem are either very long or difficult to fabricate.[17-19] We have develop an enabling technology based on a nano-taper device that allows this to be done in a very simple fashion and with very high coupling efficiency.[20]

The nano-taper consists of a waveguide laterally tapered to a nanometer-sized tip at the facet in contact with an optical fiber (Fig. 9(a)). At the tip, the field profile becomes delocalized from the waveguide core, which induces a very large mode profile similar in effective index and profile to that of the fiber. FDTD and BPM simulations show that insertion loss can be as low as 0.5dB for TE-like mode at λ_0=1550nm, this is a factor of 20 times over conventional techniques.

We have fabricated such structure shown in Fig 9(b) in Cornell. For such structure, The coupling enhancement due to the presence of the nano-taper was found to be 8 times over straight coupling to the waveguide. This was measured for both TM and TE-like modes, over the 1520-1620nm range. We estimate the insertion loss of the nano-taper at λ_0=1550nm to be 3.3±0.3 and 6.0±0.4dB for TM and TE-like modes, respectively.

The problem of extracting the signal from the receiving waveguide is reduced since we can use large area photodetectors butt-coupled to the chip with a coupler as described previously.

The high confinement Si-waveguides allow us to introduce 90o bends in the chip's passive integrated optical circuit eliminating direct coupling of laser source to the detector, simplifying design of the chip-carrier and *sensing* chip.

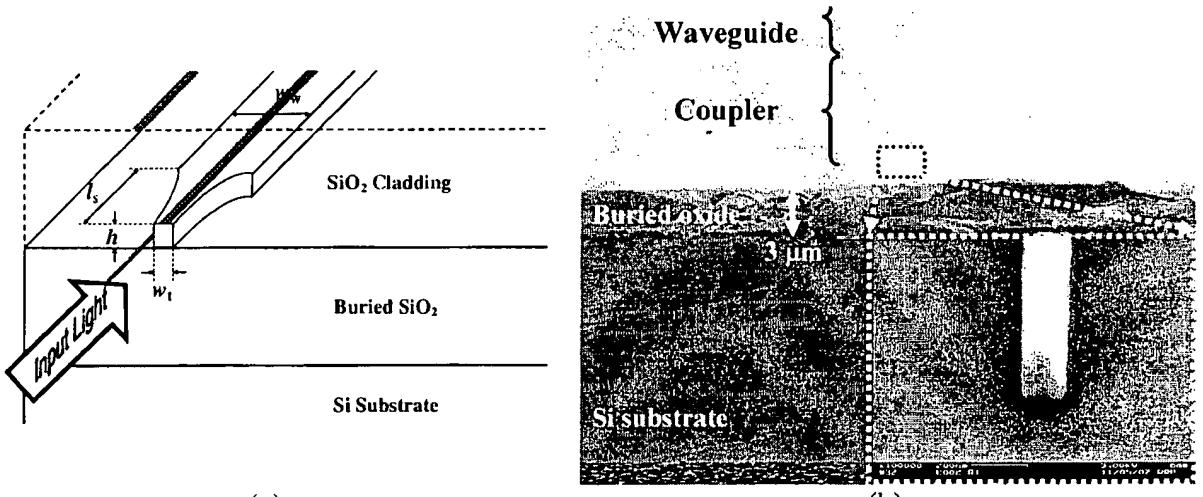


Fig. 9 – (a) Schematics of a waveguide with a nano-taper coupler. (b) Scanning electron micrograph of the cleaved edge of the wafer prior to top-cladding deposition. Inset shows a zoom of a coupler tip. (Cornell University)

The nano-taper corresponds to the shortest SOI-based mode-size converter with high coupling efficiency for bridging between optical structures across scales. The alignment accuracy needed for 3dB coupling is approximately +/- 2μm.

We plan to improve this nano-taper coupler to reduce the polarization sensitivity to TE or TM mode operation. This relaxes requirements on the light source and fiber used.

2.2.2. Arrayed Detectors by Wavelength Separation and Encoding

We intend to implement a wavelength encoded probing architecture based on ring resonators structures. Figure 11 shows a schematic of the two possible approaches we plan to investigate.

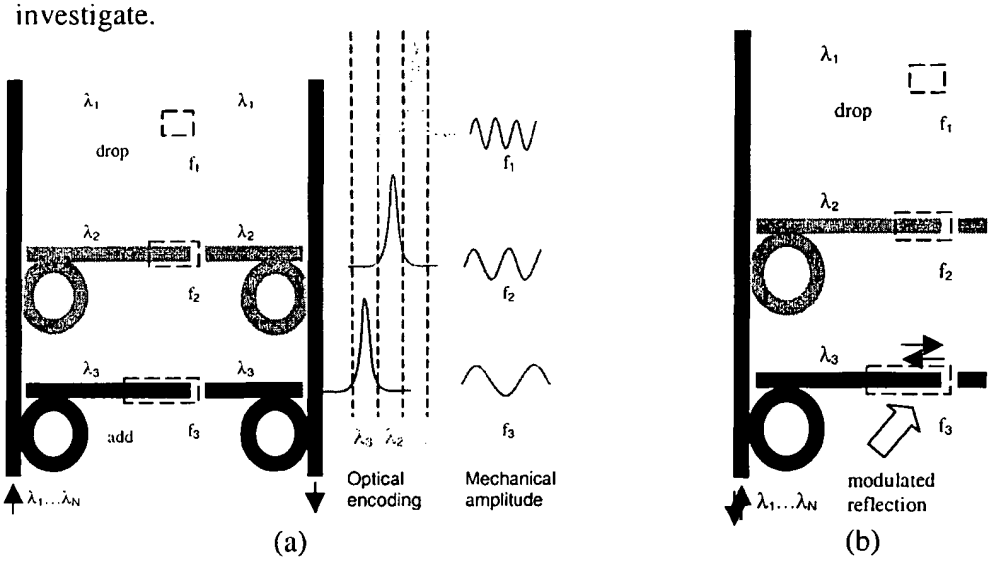


Figure 11 – (a) Diagram of Arrayed cantilevers in transmission mode. (b) Possible implementation using

reflection mode.

In Fig 11(a), the transmission of the optical signal from the cantilever waveguide to a receiving waveguide is modulated. This approach is discussed in more detail in the following section. It requires that the transmitted signal be coupled into a second waveguide through a matched ring resonator. Simply using Y-junctions would induce a 50% loss per junction, severely restricting the array size. Fig 11(a) has an insert demonstrating the spectral separation of wavelengths by the different ring resonators of different diameters. It also shows pictorially the variation in frequency of oscillation of cantilevers of different length. Note that the cantilever length does not need to be varied, as the information from each cantilever is encoded in the optical wavelength. The frequency shift which indicates positive detection can be extracted for each separate wavelength. One can use different cantilever lengths to optimize the sensitivity to the different pathogen or target masses.

In Fig 11(b), the reflection mode of operation is depicted. In this mode the optical signal reflected back into the cantilever waveguide is modulated. We plan to investigate theoretically, through simulations, the configuration that maximizes the reflected signal modulation. This method has the advantage, from an integration point of view, that only one fiber is needed to send and receive the signal. From a fabrication point of view, since the same ring resonator is used for extraction and re-insertion of the optical signal, devices do not need to be tuned. This greatly increases the yield and decreases cost.

2.2.3. Silicon waveguide cantilever - Detection of oscillation

We have demonstrated waveguides fabricated in Silicon-on-insulator (SOI) wafers having losses in the range of 3-6 db/cm which is similar to previously reported in the literature. The fabrication of such waveguides is done at the same step as the fabrication of the waveguide couplers and ring resonators described above, and the section of the waveguides that act as the cantilever. The cantilever consists of a section of a silicon waveguide suspended over the substrate. In the preferred embodiment a reflector structure faces the suspended cantilever. Alternatively, light will be collected by a fixed waveguide facing the suspended cantilever. Figure 8 describes the chip and gives the expected dimensions of the key waveguide dimensions.

Although oscillation of the cantilevers is present at all times due to thermal motion, we will greatly enhance the amplitude of the oscillations by direct drive of the chip either through on-chip electrostatic actuation or through *standard* piezoelectric driving on the chip-carrier. This will allow us to increase sensitivity until non-linearities begin to disturb the oscillation of the cantilever. For instance, piezo drive will indeed slightly degrade the quality factor (Q), and if significant drive is employed the resonance curve will topple over. We will investigate the regime of drive amplitudes (piezo and capacitive) wherein the quality factor is not significantly degraded (less than 5% drop in Q).

Initially, oscillation of the cantilever will be induced in the y-direction by a piezoelectric actuator. An initial simulation for optimized waveguide parameters in the transmission mode was performed. The results are shown in Figure 10. They yield a modulation of 10% of the light intensity with a 100 nm tip displacement in the cantilever.

We plan to develop suitable stress layers in the structure to displace the equilibrium position by 100-200 nm. This places the operation at the inflexion the curve, increasing the sensitivity. An oscillating amplitude of 20 nm yields a very large modulation of 10% in the intensity of the signal. Using sensitive electronics one can easily measure signals 10-100 times smaller than this.

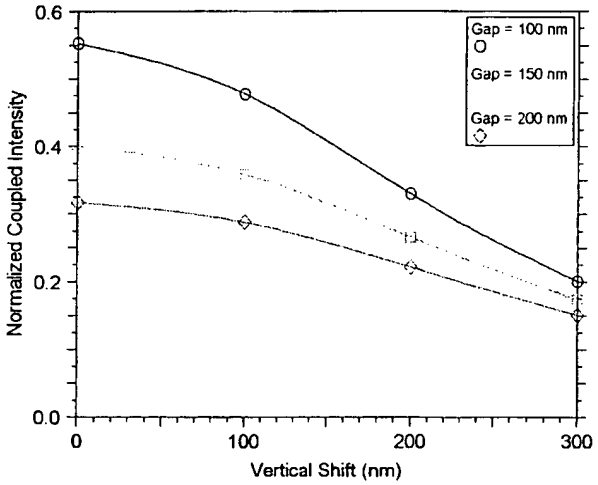


Fig. 10 – Light intensity coupled to the receiving waveguide as a function of vertical displacement of cantilever for 3 different gap sizes between cantilever and receiving waveguide.

A conservative estimate of the detection limits of the oscillation of the cantilever can be achieved through a simple calculation. We will use a 10dbm input laser signal. If we have a lower bound of –10 db coupling (misalignment of 4 μm), this means that a 0 dbm signal will reach the cantilever (or 1 mW). If we loose another 40% in coupling and achieve a 1% modulation due to oscillation, this means we have to detect a 50 μW optical signal modulated at 1 MHz.

2.2.4. Optimization of Biosensitive Site on Cantilever

2.2.4.1. Surface chemistry for immobilization

A dendrimer-based chemistry has also been synthesized to enhance biomolecular immobilization. Dendrimers are star-like in appearance and have been used to increase the active surface area for immobilization. As with the Ni-NTA chemistry, the dendrimer surface modification has demonstrated a large local binding capacity, low nonspecific binding, flexibility and long-term stability. For the majority of our studies, the dendrimer has been modified to present pendant biotin groups, thereby allowing for the immobilization of biotin-tagged molecules, with a streptavidin molecule as a bridge (Figure 12). In other studies, DNA-DNA hybridizations and other protein-protein systems have been studied using bis([sulfosuccinimidyl]suberate) (BS3) rather than biotin as linking molecule from the dendrimer surface.

We will apply dendrimer technology to modify the surfaces of the cantilever and specifically attach streptavidin. We have demonstrated the merits of dendrimer-based chemistries for performing protein-protein, protein-DNA and DNA-DNA interactions with the SR7000 [21]. Efforts will be continued in three distinct, yet related areas to further improve the construction and applicability of this chemistry. Firstly, four generation (G4) dendrimers will be investigated, thereby increasing the bioactive surface area and signal enhancement. Secondly, G4 dendrimers with mixed functionalities will be explored for extending the range of immobilization methods and orientations, and to improve the assay environment[22]. Thirdly, dendron thiols will be explored. Dendrons are tree-like molecules that contain a terminal thiol

functionality. The thiol functionality will assist in promoting orientation-specific interactions with the gold surface, and the length will be controlled for optimizing the chemical properties. If successful, the use of dendrons will reduce the current multi-step chemical synthesis to a single step.

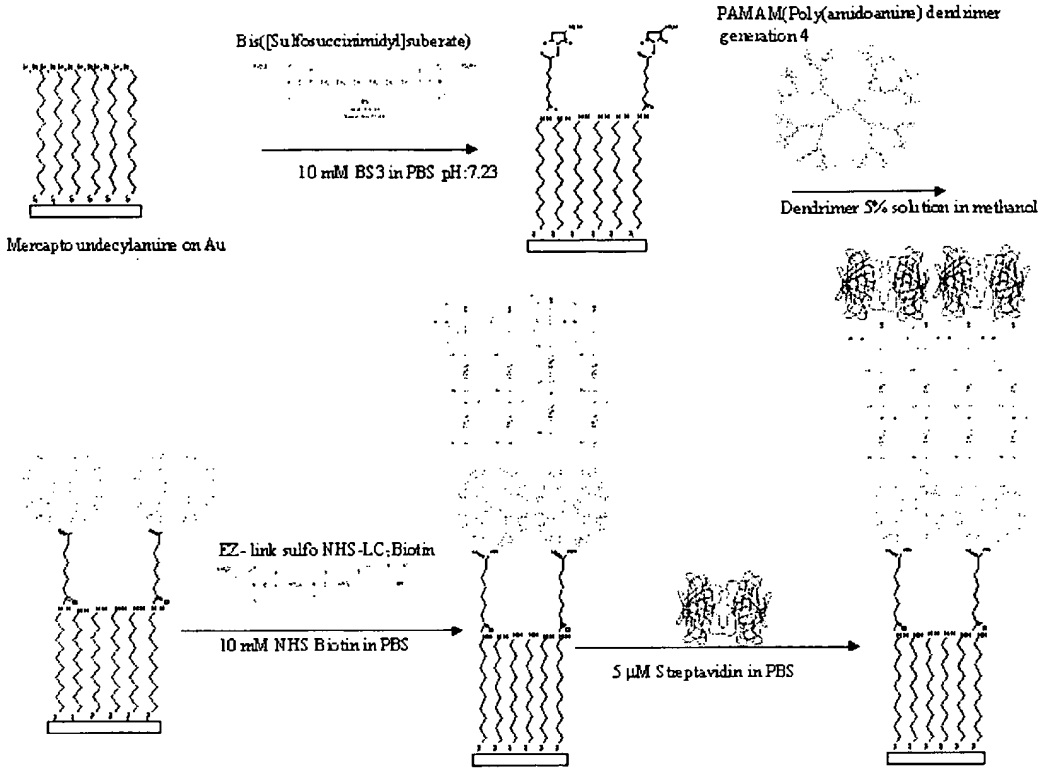


Figure 12 – Mechanism for dendrimer assisted immobilization of Streptavidin.

2.2.4.2.Biological test systems

Bacteriophage systems provide a robust and elegant means to develop unique test systems for the cantilever-based device proposed. Phage display systems for M13, T7 and lambda have been developed and each offers the opportunity to express on the surface unique peptides with a wide variety of binding properties. In these display systems, a library of protein or peptide variants are generated and exposed on the surface of either a cell or a phage. This population can then be screened for binding to particular target and those that bind physically separated from the remaining population. Those cells, or phage that bind to the target can then be biologically amplified and the process repeated. Through a series of 'pannings', proteins or peptides that have an ever-increasing ability to bind to the target are therefore selected. The selection and amplification process mimic evolution and orders of magnitude increase in binding affinity are realized.

In phage display a library of peptides or larger molecules (including Fab or scFv) are represented within a population of bacteriophage which express them on the surface. One of the most commonly used phage display systems is the filamentous virus M13 with the displayed

peptides fused to the gpIII coat protein. Effectively over 10^6 different variants can be displayed and screened to select for peptides that bind to the target antigen of interest. In a manner similar to clonal propagation of antibody producing cells, phage which express a peptide on their surface that has superior binding properties can be removed from solution by immobilization of the antigen. Once bound, the remainder of the phage can be removed by washing, leaving behind the selected phage. Bound phage can then be eluted and more importantly propagated to enrich for phage that have displayed proteins with superior binding properties. This process of biological panning, *i.e.,* biopanning, can be repeated in a cyclical fashion, each step enriching for the phage that express on their surface peptides with ever increasing ability to bind to the target antigen. The displayed peptide can be easily manipulated and examined to determine its sequence. We have in fact already isolated M13 phage that have a consensus sequence that behaves like biotin, it is able to bind to streptavidin with a high affinity. Its sequence is in agreement with previous reports [23]

Bacteriophage T7 and M13 will be used as test systems each of which selected for their display of a 'biotin-like' peptide. These will provide at least two different biological test systems of different mass. While the individual variation in mass of M13 or T7 is not known, it is likely that the population is variable depending upon the number of coat proteins molecules incorporated into the capsid. This natural variation will provide a good test for the cantilever system.

3. Fabrication

Standard microfabrication techniques available in the microelectronics industry are quickly approaching the requirements for fabrication of the proposed device. Current microprocessors already fabricate features in the 130 nm scale, while our technology requires dimensions in the 80-450nm. According to Intel's lithography roadmap, a features size of 90 nm will be in production this year.[24] In the near future, specific sensors developed for widespread use in pathogen detection can be mass produced in a silicon platform.

The key fabrication process steps we plan to use in the fabrication of the device are described in Figure 13. The release of the cantilever will pose unique challenges as we must make sure that the receiving waveguide will not oscillate during operation. This is necessary as otherwise this may preclude a direct measurement of the cantilever's oscillating frequency, as the output will contain components from the oscillation of the receiving waveguide as well.

The fabrication of integrated optic structures, especially waveguides, in silicon requires smooth sidewalls to achieve low loss. The smallest dimensions used in our devices are found in the nano-taper coupler and in the gap between the cantilever and receiving waveguide. They are beyond the capabilities of the standard *photolithography* equipment available at the Cornell Nanofabrication Facility (CNF). The patterning of our device requires use of *state-of-the-art electron-beam lithography* at the CNF. Patterning is one of the key steps in the process of the proposed device.

Currently, site specific binding is achieved on cantilevers that undergo minimal processing for their fabrication, and therefore we must verify that any additional processing steps needed to complete our chip do not interfere with this process and are fully compatible with that biochemistry.

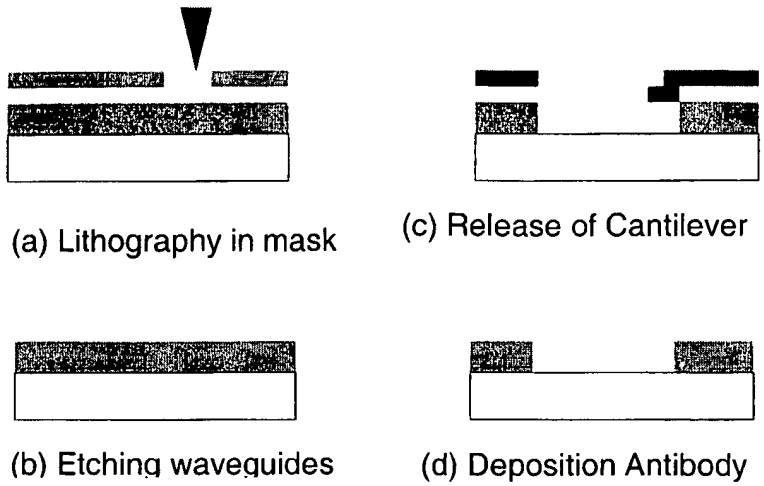


Figure 13 – Fabrication sequence containing the key steps in the process flow. (a) Electron-beam lithography; (b) Etching of waveguide/coupler/cantilever; (c) Protection of fixed waveguides; (d) Release of cantilever and deposition of biosensitive antibody layer.

4. Milestones

The development of a full prototype requires the demonstration of the following key enabling steps: Initially we will investigate design of the device for optimal performance through detailed simulations. Secondly, we will evaluate potential fabrication processes for integrating the suspended waveguide cantilever and the fixed receiving waveguide or reflecting element. We will then evaluate the dynamic performance of the device under induced oscillation.

Once this is demonstrated we will proceed to investigate a compatible approach with the biosensitization of site specific binding. At this stage we will demonstrate an initial prototype with nano-mechanical waveguide chip used in detection of biomass.

In parallel, we will investigate novel immobilization techniques that will allow us to optimize the process of deposition of antibodies in a way consistent with the fabrication process for such nano-opto-mechanical structures.

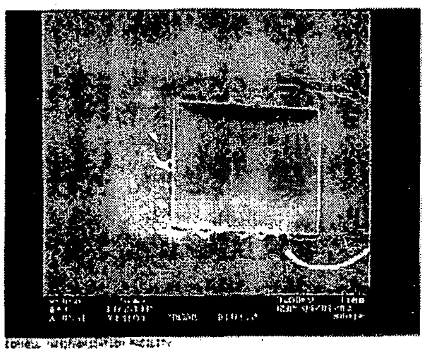
EXHIBIT

B

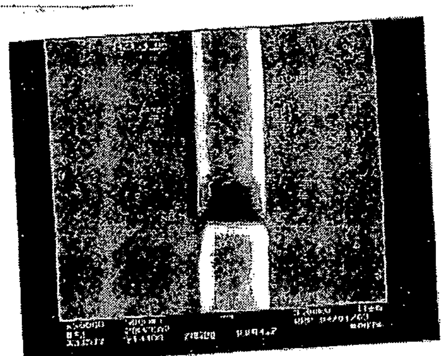
4. Fabrication results fo

Project: Alignment mask - Beam inspection

Notebook Number:



CORNELL NANOFABRICATION FACILITY



CORNELL NANOFABRICATION FACILITY

Gap - initial
tests on
cavity + qdot.

Signature

Date

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Date

EXHIBIT C

Cornell University Invention Disclosure

Submit Completed Form To:

CORNELL RESEARCH FOUNDATION, INC.
20 Thornwood Drive, Suite 105
Ithaca, New York 14850 (or campus mail)
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Docket No. 3332
Technology No. EN927
Classification Code:____
Date Received:_____

Title of Invention

Integrated optical waveguides for sensing of displacement

We propose the use of integrated optical waveguides acting as cantilever sensors in optical circuits. Using special waveguides acting as cantilevers enables optical detection of deflection/displacement amplitude, including oscillations. Detection of mass selectively attached to a cantilever is achieved by means of the measurement of a change in resonant frequency of mechanical oscillation. More generally, the displacement of the cantilever can be sensed optically, enabling all-optical and self-aligned detection of integrated cantilevers.

Brief Description of Invention

Cantilever beams micro-machined in silicon are currently used as displacement sensors in such devices as atomic force microscopes, magnetic force microscopes and other force based scanning probe techniques where force applied to the cantilever causes a physical displacement is transduced by some means and detected. Cantilever beams are also used to detect mass, since the mechanical oscillation of such a structure presents a resonant frequency which is a function of the mass attached to the cantilever, in addition to the mass of the cantilever itself. Coupled to a suitable test mass, inertial forces can also be sensed by measuring displacement.

To date cantilever detection has been achieved by means of one of: 1) deflection of external laser beam reflecting off of the cantilever [AFM]; 2) piezoresistive measurements of special cantilever materials [e.g., Haronian, D.]; 3) capacitance change between cantilever and substrate; 4) measurement of tunneling current [STM]. These techniques suffer from disadvantages relating to their use as sensitive mass sensors due to: 1)complex mounting; 2) lack of sensitivity for submicron cantilevers; 3) lack of sensitivity for submicron cantilevers; 4) difficult fabrication and too much environmental instability. Finally, optical measurements enable sensing in areas or applications where large electromagnetic interference precludes electrical measurements such as those described above.

We have identified optically transparent materials, such as silicon and silicon nitride, that in addition to supporting waveguiding, can serve as cantilever materials. Silicon has been shown to be a very good optical medium, in special for wavelengths between 1.2 um-1.7um. Silicon waveguides fabricated in silicon-on-insulator structures have already been demonstrated in suspended waveguide applications.

Theoretical analysis of suspended waveguides acting as cantilevers show that both the optical transmission and reflection are affected by displacement of the cantilever/waveguide. Transmission from the cantilever waveguide can be made from the suspended facet into an anchored waveguide. Reflection of light exiting the cantilever waveguide back into the same waveguide can be achieved by a suitable reflector material placed some distance to the facet of the waveguide.

For further information please see attached copy of proposals to NIH and NSF:

1) NIH - PAR-03-045: NANOSCIENCE AND NANOTECHNOLOGY IN BIOLOGY AND MEDICINE
Submitted 02/18/2003
Integrated Optical Detection of Nanoscale Biomass
Dr. Roberto Panepucci – PI, Prof. Michal Lipson – Co-PI, Prof. Carl Batt – Co-PI

2) NSF 03-512 - Program Solicitation
SENSORS AND SENSOR NETWORKS
Submitted 03/06/2003
Sensors: Arrayed Optical Detection of Nanoscale Biomass
Prof. Michal Lipson, PI, Dr. Roberto Panepucci, Co-PI, Prof. Carl Batt, Co-PI.

PLEASE INCLUDE COPIES OF RELEVANT REFERENCES WITH THIS INVENTION DISCLOSURE

Sponsorship

US Government _____ Commercial/Private _____ Cornell Univ. X Personal _____ Biotech _____ Other_____

Name of Sponsor	Sponsor's Contract No.	Cornell Univ. Account No.

Q: Was this invention developed using resources in the Theory Center or the Biotech Building? N (Y/N)

Collaborating Institution / Company/Organization

Organization	Principal Investigator Name	Address

Record of Invention

Date of Conception [redacted] Documented: Yes [ ] No [X] Form _____ Location _____

Invention Reduced to Practice: Yes [X] No [ ] Date of First Reduction to Practice [redacted] Prototype Available : Yes [ ] No [X]

Publication(s)

Submittal: Date _____ Journal _____ Publication Date _____ [ ] Estimated [ ] Actual

Oral Disclosure: Date _____ Occasion _____ Handouts: [ ] Yes [ ] No

Poster presentation: Date made _____ Published abstract: [ ] Yes [ ] No Citation _____

Commercial Interest

Company Name	Location	Contact Person	Title

Inventor Data (1)

Name Roberto Ricardo Panepucci Campus Address/Dept 212 Phillips Hall

Home Address 600 Warren Road Ap 4-1A Title Senior Researcher Associate

City /ZIP Ithaca, NY 14850 Citizenship USA Social Security No. 220-27-5905

Email panepucci@cnf.cornell.edu Home Phone (607) 592-5633 Campus/Office Phone (607) 255-7189 FAX (607) 254-3508

Inventor Data (2)

Name Bojan Illic Campus Address/Dept 126 Phillips Hall

Home Address 145 Coddington road Apt#1b Title Title Research Support Specialist II

City /ZIP Ithaca, NY 14850 Citizenship Yugoslavian Social Security No. 321-74-6896

Email rob@cnf.cornell.edu Home Phone (607)257-9666 Campus/Office Phone 5-2329x119 FAX 5-8601

Inventor Data (3)

Name Michal Lipson Campus Address/Dept. 411 Phillips Hall

Home Address 641 Highland Rd Title Assistant Professor

City /ZIP Ithaca, NY 14850 Citizenship USA Social Security No. 019-82-7839

Email mlipson@ece.cornell.edu Home Phone (607) 257-6607 Campus/Office Phone (607) 255-7877 FAX 607) 254-3508

Inventor Data (4)

Name Vilson Rosa de Almeida Campus Address/Dept. 429 Phillips Hall

Home Address 910 Hasbrouck Apts Title PhD Student

City /ZIP Ithaca, NY 14850-2657 Citizenship Brazil Social Security No. 068-90-7165

Email vra2@cornell.edu Home Phone (607) 253-6578 Campus/Office Phone (607) 255-9609 FAX (607) 254-3508

Name of Primary Contact: Roberto Panepucci

Review / Approval (Optional Depending on Department Policy)

Dean / Chair /Director Name_____	_____Signature_____	_____ Date_____

Inventor Signature(s): Per Cornell University Patent Policy, I (We) hereby assign all right, title, and interest in and to this invention to Cornell Research Foundation, Inc. ("CRF") and agree to execute all documents as requested to assign my (our) rights to CRF in and to any patent application or other statutory form of intellectual property protection filed on this disclosure, and to cooperate with CRF in securing protection of the disclosed invention. CRF will share any income derived from this invention with the Inventor(s) recorded hereon and the Inventor(s) department(s) according to its published policies.

Inventor Signature	Date	Witness Signature	Date
(1)			
(2)	[redacted]		[redacted]
(3)			
(4)			